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Molecular evidence for the loss of three basic tastes in penguins

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Sensing its biotic and abiotic environmental cues is critical to the survival and reproduction of any organism. Of the five traditionally recognized senses of vertebrates, taste is dedicated to the differentiation between nutritious and harmful foods, triggering either appetitive or rejective behaviors [1]. Vertebrates typically can detect five basic taste qualities: sweet, umami, bitter, sour and salty. Remarkable progress in understanding the molecular basis of taste [1] has opened the door to inferring taste abilities from genetic data. Based on genome and relevant gene sequences, we infer that the sweet, umami, and bitter tastes have been lost in all penguins, an order of aquatic flightless birds originating and still occupying the coldest ecological niche on Earth, the Antarctic [2].

Vertebrate tastes are mediated by taste receptors. The candidate receptors for sour and salty tastes are transient receptor potential ion channel PKD2L1 and sodium channel ENaC, respectively [1]. Umami and sweet tastes are sensed by the Tas1r family of G protein-coupled receptors (GPCRs), with the Tas1r1–Tas1r3 heterodimer functioning as the umami receptor and the Tas1r2–Tas1r3 heterodimer being the sweet receptor [1]. Bitter tastants are detected by the Tas2r family of GPCRs [1].

We started by analyzing high-coverage genome sequences of Adelie penguin (*Pygoscelis adeliae*), emperor penguin (*Aptenodytes forsteri*), and little egret (*Egretta garzetta*), and publically available genome sequences of 13 other birds (Figure 1). Both Adelie and emperor penguins inhabit Antarctica, while the little egret, belonging to Ciconiiformes, represents a relatively

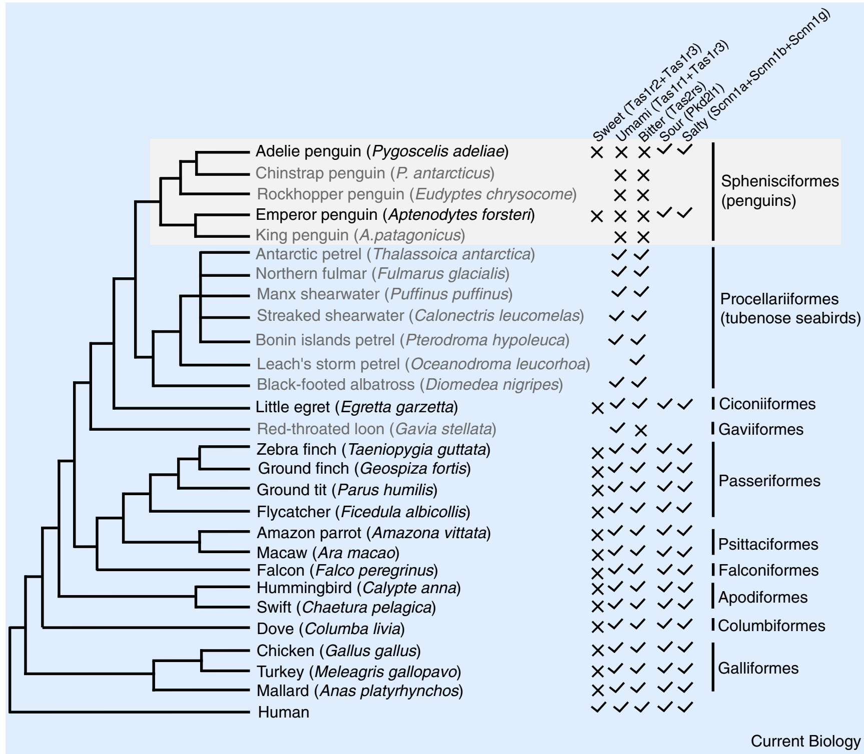

Figure 1. Taste loss in penguins.
Species tree of 27 birds and human showing presence (check marks) and absence (crosses) of sweet, umami, bitter, sour, and salty tastes in penguins (shaded) and outgroups, inferred from genes for taste receptors (shown at the top of the figure). Neither check mark nor cross is given to a species when there is no genetic/genomic data for such an inference. Species with available genome sequences are shown in black, whereas those without available genome sequences are shown in grey. The red-throated loon *Gavia stellata* is considered a putative bitter non-taster due to the pseudogenizations of *Tas2r1* and *Tas2r2* that are independent from the penguin-specific pseudogenizations. Note that the umami taste receptor has been repurposed to detect sweet in the hummingbird *Calypte anna* [10]. As a result, *C. anna* possesses the sweet taste, in addition to a weak umami taste. See also Supplemental information.

close outgroup of penguins. We failed to identify the sweet taste-specific receptor gene *Tas1r2* in any of the 16 bird genomes (Figure 1), although *Tas1r2* was identified in the genomes of mammals, reptiles and other vertebrates by the same approach.

We similarly searched for the umami taste-specific receptor gene *Tas1r1*. In all non-penguin bird genomes examined, *Tas1r1* has an intact open reading frame (ORF) (Figure 1), but in the two penguins, *Tas1r1* is a pseudogene characterized by a common two base-pair deletion that results in premature stop codons (Supplemental Information). We examined three additional penguin species, one species in Gaviiformes, and seven species in Procellariiformes (tubenose seabirds); the latter order is the closest outgroup of penguins (Figure 1). The additional sequences (Supplemental Information) confirmed that *Tas1r1* was pseudogenized in the common ancestor of all penguins since its separation from tubenose seabirds.

We next examined *Tas1r3*, encoding the shared component of the sweet and umami receptors. We found an intact *Tas1r3* gene in each non-penguin bird genome examined, but failed to detect *Tas1r3* in the two penguin genomes despite the presence of their neighboring genes. Examining the 11 additional birds mentioned before confirmed that *Tas1r3* was lost in the most recent common ancestor of all extant penguins (Figure 1 and Supplemental Information).

We detected one to seven intact *Tas2r* bitter taste genes from each of the 14 non-penguin bird genomes (Supplemental Information). Of note, the little egret has two intact

*Tas2r*s and one pseudogene. Strikingly, in each penguin genome, all we could find were three *Tas2r* pseudogenes that are the respective orthologs of the three little egret *Tas2r*s (Supplemental Information). This finding, combined with additional *Tas2r* data collected from some of the aforementioned 11 birds (Supplemental Information) demonstrates that the entire *Tasr2r* repertoire was pseudogenized in the common ancestor of penguins (Figure 1).

Trpm5 and Calhm1 are indispensable for umami, sweet, and bitter taste transduction [3,4]. Our evolutionary analysis of *Trpm5* and *Calhm1* sequences revealed relaxations of purifying selection in penguins (Supplemental Information). Furthermore, ORF-disrupting mutations in *Calhm1* were found in three penguins (Supplemental Information).

By contrast, the putative receptor gene for the sour taste, *Pkd2l1*, was identified in each of the 16 bird genomes (Figure 1). Evolutionary analysis suggests that *Pkd2l1* is under purifying selection in all birds. A similar result was found for *Scnn1a*, *Scnn1b* and *Scnn1g* that encode the three subunits of the putative salty taste receptor ENaC (Figure 1).

These results suggest that penguins can perceive salty and sour tastes, but because these receptors may have other functions and because not all receptors for these two tastes are known, a behavioral test will be needed to validate this prediction.

Taken together, our results strongly suggest that the umami and bitter tastes were lost in the common ancestor of all penguins whereas the sweet taste was lost earlier. Although behavioral tests of penguin tastes are lacking, an anatomical study showed no taste buds in the tongues from four penguin species [5]. Because taste buds are the primary locations of taste receptor cells, the lack of taste buds strongly suggests a reduction in taste function.

Furthermore, penguin tongues possess only a single type of lingual papillae that are stiff, sharp, and caudally-directed, and the numerous papillae are all covered by a thick cornified layer, suggesting that penguin tongues are used primarily for catching and holding slippery fishes or other prey [5].

Why are the sweet, umami, and bitter tastes, especially the latter two, dispensable in penguins? Given that penguins are carnivorous, it seems unlikely that they would not require umami taste. But their behavior of swallowing food whole and their tongue structure and function suggest that penguins need no taste perception, although it is unclear whether these traits are a cause or consequence of their major taste loss.

It is intriguing to note that Trpm5, required for transducing the sweet, umami, and bitter tastes but not sour or salty taste [4], is temperature-sensitive, with lower activities at lower temperatures [6]. Trpm5 may be effectively non-functional in ancestral penguins' taste buds (likely ~0°C), rendering the tastes that rely on this channel unusable and gradually lost. Furthermore, adaptation of Trpm5 to a low temperature might have been prohibited because Trpm5 also performs non-taste functions [7,8] in the body (***e.g.***, 39°C in emperor penguins). In the future, it would be interesting to test the hypothesis that failure to resolve Trpm5's antagonistic pleiotropy [9] in the face of an extremely cold environment caused the major taste loss in penguins.

It is noteworthy that, although penguins are not limited to the Antarctic, they originated in the Antarctic. Recent molecular dating suggested that the extant penguins radiated after the formation of large ice sheets. If ancestral penguins had lost the receptor genes for the three tastes while in the Antarctic, the genes and the tastes cannot be regained even when some penguins migrated out of the Antarctic. Considering such historical contingencies is important when making sense of the relationship between the feeding ecology and taste ability among species.

Supplemental Information

Supplemental Information including supplemental results, discussion and experimental procedures and one figure and one table can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.01.026.

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